

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Terry et al.

Group Art Unit: 1638

Serial No. 09/365,348

Examiner: Ibrahim, M.

Filed: July 30, 1999

For: *Trace Element Phytoremediation*

Attorney Docket No. B99-084

## DECLARATION UNDER RULE 132

I, Lewis Feldman, declare and state as follows:

1. I am a Professor in the Department of Plant and Microbial Biology at the University of California, Berkeley. The Regents of the University of California is the assignee of the subject patent application. I am knowledgeable and experienced in the field of genetic engineering in plants. I have read and am familiar with the contents of the above application.

2. The product claims of this application are drawn to a plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provides enhanced heavy metal accumulation as compared with a corresponding wild type plant. The corresponding method claims require only two steps (a) identifying a medium as containing an excessive amount of a heavy metal; and (b) growing a subject plant in the medium, under conditions wherein the glutamylcysteine synthetase is overexpressed, whereby the plant provides enhanced accumulation of the heavy metal, whereby the heavy metal content of the medium is decreased.

The specification teaches that "a wide variety of plants may be used, as urged by the particular trace element, medium, site geology, topology, weather, etc. Additional factors for selection include large biomass production, relatively high trace element accumulation capacity, and ease of genetic engineerability", citing Zhu et al., 1999, *Plant Physiol* 119:73-79. Specification, p.4, lines 6-9. The claims are structurally limited to a plant genetically engineered to overexpress glutamylcysteine synthetase and functionally limited to one which does in fact overexpress the recited glutamylcysteine synthetase *and* thereby provides enhanced accumulation of the targeted heavy metal as compared with a corresponding wild type plant (see claim 1).

"Suitable plants are readily screened for requisite engineerability and expression from exemplars of candidate plant varieties by those skilled in the art of plant genetic engineering, as exemplified below." Specification, p.4, lines 9-11. The specification offers a large number of suitable, commercially available varieties of exemplary plant source materials (p.4, line 11 - p.6, line 9). Furthermore, the specification describes diverse exemplary plant species demonstrating enhanced elemental assimilation in wild-type plants and the corresponding plant overexpressing a variety of recombinant glutamylcysteine synthetase genes (p.7, line 26 - p.8, line 18); exemplified plants include Brassica juncea, Populus angustifolia, Nicotiana tabacum and Silene cucubalis. The suitability of any given plant is readily ascertained by simple substitution into the same method.

The invention is premised on Applicants' finding that the recited glutamylcysteine synthetase effects heavy metal accumulation, is causative of heavy metal accumulation and is rate-limiting of heavy metal accumulation. The disclosure establishes a predictable relationship between heavy metal exposure and overexpression of glutamylcysteine synthetase; namely, that such overexpression promotes enhanced accumulation of the metal. This relationship is shown to hold across numerous and diverse exemplary plant species (supra). Accordingly, as an expert in the field, it is my opinion that the specification aptly enables one of ordinary skill in the art to practice the method in any plant which is genetically engineered to overexpress glutamylcysteine synthetase and thereby provide enhanced accumulation of the heavy metal.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: \_\_\_\_\_

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Prof. Lewis Feldman